

CLAIMS

1. A substantially planar substrate for use in patch clamp analysis of the electrophysiological properties of a cell membrane comprising a glycocalyx, wherein the substrate comprises an aperture having a rim defining the aperture, the rim being adapted to form a gigaseal upon contact with the cell membrane, the rim protruding from the plane of the substrate to a height in excess of the thickness of the glycocalyx.
2. A planar substrate according to Claim 1 wherein the rim protrudes from the plane of the substrate to a height of at least 20 nm above the surface of the planar substrate, preferably least 30 nm, at least 40 nm, at least 50 nm, at least 60 nm, at least 70 nm, at least 80 nm, at least 90 nm or at least 100 nm.
3. A planar substrate according to any one of the preceding claims wherein the width of the rim is in the range 50 to 200 nm.
4. A planar substrate according to any of the preceding claims, in which the length (i.e. depth) of the aperture is between 2 and 30  $\mu\text{m}$ , preferably between 2 and 20  $\mu\text{m}$ , 2 and 10  $\mu\text{m}$ , or 5 and 10  $\mu\text{m}$ .
5. A planar substrate according to any of the preceding claims wherein the diameter of the aperture is in the range 0.5 to 2  $\mu\text{m}$ .
6. A planar substrate according to any one of the preceding claims wherein the rim extends substantially perpendicularly to the plane of the substrate.

38

7. A substrate according to any one of Claims 1 to 5 wherein the rim forms an oblique angle with the plane of the substrate.

8. A substrate according to any one of Claims 1 to 5 wherein the rim is substantially parallel to the plane of the substrate.

9. A substrate according to Claim 1 wherein the rim is defined by a mouth of the aperture, which mouth has a radius of curvature between 5 and 100nm with an angle of 45 to 90 degrees.

10

10. A planar substrate according to any of the preceding claims wherein the substrate is made of silicon, plastics, pure silica or other glasses, such as quartz and Pyrex<sup>TM</sup>, or silica doped with one or more dopants selected from the group of Be, Mg, Ca, B, Al, Ga, Ge, N, P, As.

15

11. A planar substrate according to Claim 10 wherein the substrate is made of silicon.

12. A substrate according to any of the preceding claims wherein the surface of the substrate and/or the walls of the aperture are coated with a second coating material.

13. A substrate according to Claim 12 wherein the coating material is silicon, plastics, pure silica, other glasses such as quartz and Pyrex<sup>TM</sup>, silica doped with one or more dopants selected from the group of Be, Mg, Ca, B, Al, Ga, Ge, N, P, As, or oxides of the same.

25

14. A substrate according to Claim 10 wherein the coating material is silicon oxide.

30

39

15. A method of making a substantially planar substrate for use in patch clamp analysis of the electrophysiological properties of a cell membrane comprising a glycocalyx, wherein the substrate comprises an aperture having a rim defining the aperture, the rim being adapted to form a gigaseal upon contact with the cell membrane, the method comprising the steps of

(i) providing a substrate template;

(ii) forming an aperture in the template; and

10

(iii) forming a rim around the aperture such that the rim protrudes from the substrate to a height in excess of the thickness of the glycocalyx.

15 16. A method according to Claim 15 wherein the substrate is manufactured using silicon micro fabrication technology.

17. A method according to Claims 15 or 16 wherein step (ii) comprises forming an aperture by use of an inductively coupled plasma (ICP) deep reactive ion etch process.

20

18. A method according to any one of Claims 15 to 17 further comprising the step of coating the surface of the substrate.

25 19. A method according to Claim 18 wherein step (iii) is performed at the same time as coating the substrate.

20. A method according to Claim 18 wherein step (iii) comprises an intermediate step of a directional and selective etching of the front side of the substrate causing a removal of a masking layer on the front side of the

30

40

substrate, and further proceeding the prescribed protrusion distance into the underlying substrate.

21. A method according to Claims 18, 19 or 20 wherein the coating is deposited by use of plasma enhanced chemical vapour deposition (PECVD) and/or by use of low pressure chemical vapour deposition (LPCVD).

22. A method according to Claim 21 wherein the coating is deposited by use of plasma enhanced chemical vapour deposition (PECVD).

10

23. A method according to Claim 17 wherein step (iii) comprises forming a rim from a deposited surface coating by use of plasma enhanced chemical vapour deposition (PECVD).

15 24. A method for analysing the electrophysiological properties of a cell membrane comprising a glycocalyx, the method comprising the following steps:

20 (i) making a substantially planar substrate for use in patch clamp analysis of the electrophysiological properties of a cell membrane comprising a glycocalyx, wherein the substrate comprises an aperture having a rim defining the aperture, the rim being adapted to form a gigaseal upon contact with the cell membrane, the method comprising the steps of

25

(ii) providing a substrate template;

(iii) forming an aperture in the template; and

30

41

(iv) forming a rim around the aperture such that the rim protrudes from the substrate to a height in excess of the thickness of the glycocalyx.

5 (v) contacting the cell membrane with the rim of an aperture of the substrate such that a gigaseal is formed between the cell membrane and the substrate; and

10 (vi) measuring the electrophysiological properties of the cell membrane.

25. A kit for performing a method according to Claim 24, the kit comprising a substantially planar substrate for use in patch clamp analysis of the electrophysiological properties of a cell membrane comprising a  
15 glycocalyx, wherein the substrate comprises an aperture having a rim defining the aperture, the rim being adapted to form a gigaseal upon contact with the cell membrane, the rim protruding from the plane of the substrate to a height in excess of the thickness of the glycocalyx and one or more media or reagents for performing patch clamp studies.

20

26. A substrate substantially as herein before described with reference to the accompanying drawings.

25 27. A method substantially as herein before described with reference to the accompanying drawings.

28. A kit substantially as herein before described with reference to the accompanying drawings.